PREVALENCE OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE VARIANTS AMONG MALARIA PATIENTS IN A MALARIA-ENDEMIC AREA ALONG THAI-MYANMAR BORDER

Jiraporn Kuesap[1], Pantip Kittipreechakul[1], Napim Naowaratkulchai[1], Wanna Chaijaroenkul[2], Kesara Na-Bangchang[2, *]

[1] Graduate Program in Biomedical Sciences, Faculty of Allied Health Sciences, Thammasat University, Pathumthani, 12120, Thailand; [2] Graduate Program in Bioclinical Sciences, Chulabhorn International College of Medicine Thammasat University, Pathumthani, 12120, Thailand

ABSTRACT:

Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is relatively common in malaria-endemic areas. This deficiency can lead to hemolysis and impact on malarial treatment with primaquine. The objective of this study was to examine the prevalence of G6PD variants in malaria patients living in malaria endemic regions along the Thai-Myanmar border.

Methods: One hundred and nine dried blood spot samples were collected from malaria clinics in Mae Hong Son, Tak, and Ranong provinces during 2011 to 2013. Three variants of G6PD mutation, i.e., Mahidol, Viangchan, and Chinese 4, were detected by polymerase chain reaction (PCR).

Results: Only Mahidol variant was found with the frequency of 9.17% (10/109), 1 in Thailand (4.17%) and 9 in Myanmar (10.58%).

Conclusions: The frequency of G6PD Mahidol variants found in one hundred and nine patients was associated with ethnicity. This may have an impact on treatment with primaquine and other 8-aminoquinoline antimalarial drugs.

Keywords: Glucose-6-phosphate dehydrogenase deficiency, Mahidol variant, Malaria, Thai-Myanmar border

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INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a hereditary abnormality in the activity of glucose-6-phosphate dehydrogenase enzyme. G6PD deficiency affects more than four-hundred million people around the world. Glucose-6-phosphate dehydrogenase enzyme reduces NADP to NADPH, while oxidizing glucose-6-phosphate. NADPH is used by the cell for reductive reactions and protects against oxidation [1]. G6PD variants were classified by World Health Organization to five groups according to enzyme activity: Class I (severe deficiency of the enzyme with chronic nonspherocytic > haemolytic anaemia), Class II (severe deficiency with enzyme activity < 10% of normal), Class III (moderate deficiency with enzyme activity 10-60% of normal), Class IV (very mild to none deficiency with enzyme activity 60-100% of normal), and Class V (increased enzyme activity) [2]. Single nucleotide polymorphism (SNP) in G6PD gene located on the X chromosome leads to the reduction of enzyme activity in hemizygous males and homozygous females [3]. These variants are associated with degree of haemolysis in different populations. G6PD deficiency is spatially distribution across malaria endemic regions where primaquine is an important standard drug against infectious *Plasmodium falciparum* gametocytes and relapsing *Plasmodium vivax* [4-6]. Moreover, primaquine is the only drug that has an activity to against the transmission of all *Plasmodium* species [7]. However, Primaquine can cause acute hemolysis in G6PD deficiency patients [8]. The clinical symptoms

[*] *Correspondence to: Kesara Na-Bangchang*
E-mail: kesaratmu@yahoo.com.

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Table 1 Restriction enzymes used and G6PD mutation variants in the study samples

G6PD variant	Nucleotide substitution	Restriction enzyme	Restriction size (bp)
Mahidol	487 G > A	HindIII	N 104, M 82+22
Viangchan	871 G > A	XbaI	N 126, M 106+20
Chinese 4	392 G > T	BstEII	N 188+15, M 203

N represents normal variant
M represents mutant variant

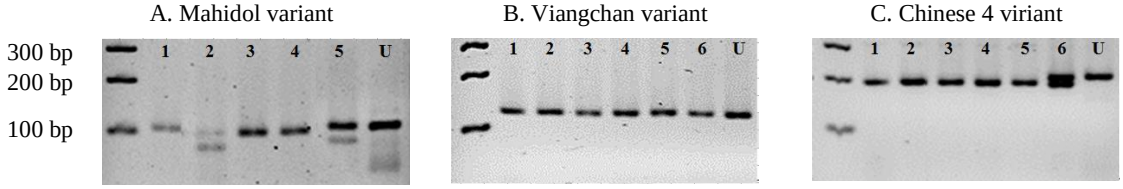

Figure 1 Genetic polymorphism of G6PD variants. (A) Mahidol variant: lanes 1, 3, and 4 represent normal variant; lane 2 represents mutant variant; lane 5 represents control for Mahidol variant; and lane U represents uncut PCR with 104 bp (B) Viangchan variant: lanes 1-6 represent normal variant and lane U represents uncut PCR with 126 bp; Chinese 4 variant: lanes 1-5 represent normal digestion; lane 6 represents control for Chinese 4 variant, and lane U represents uncut PCR with 203 bp.

caused by primaquine-induced haemolysis range from inappreciable to lethal symptoms depending on the variants of G6PD deficiency and dosing of primaquine [9]. At least 186 mutations have been characterized in the G6PD gene [10]. There are three common variants in which the primaquine sensitivity phenotypes have been characterized, i.e., G6PD A (common in Africa), G6PD Mediterranean (common in western Asia), and G6PD Mahidol (common in Southeast Asia and very common among communities in Myanmar and Thai populations) [11, 12]. In Southeast Asia, the predominant G6PD deficient variants have been reported from different populations. G6PD Viangchan and G6PD Mahidol are most prevalent in Thailand [13, 14]. G6PD-Mahidol is the dominant mutation in Burmese population [15, 16]. G6PD Viangchan is the most common variant in Laotians [15], Malaysian Malays [17, 18], Cambodians [19], and Vietnamese [20]. In Thailand, primaquine was used for treatment of both *P. falciparum* (transmitted prevention) and *P. vivax* (erythrocytes stage eradication). The prevalence of *P. vivax* has been increasing in Thailand and this might have an impact on the treatment of G6PD deficiency patients with primaquine or other 8-aminoquinolines. The aim of the present study was to examine the prevalence of G6PD variants in malaria patients living in malaria endemic regions of Thailand along the Thai-Myanmar border.

METHODS

Study subjects and sample collection

A total of 109 finger-prick blood samples (50 µl) were collected onto a filter paper (Whatman No. 3 filter paper) from malaria patients (24 Thais and 85 Burmeses; 77 *P. vivax* and 32 *P. falciparum*) who visited malaria clinics in Mae Hong Son (n=19), Tak (n=70), and Ranong provinces (n=20) during 2011 and 2013. Giemsa-stained thin and thick blood smears were prepared and examined microscopically for the presence of malaria parasites. All samples were randomly collected for genotyping of G6PD variants without any screening of G6PD deficiency. The procedure of collecting human blood samples in this study was approved by the Human Ethical Review Board of Thammasat University (no. 040/2555).

Parasite genomic DNA extraction

Each dried blood spot sample was cut and total genomic DNA was extracted using QIAamp DNA extraction mini-kit (QIAGEN, CA, USA) and stored at −20°C until used.

G6PD variants analysis

Three variants of G6PD mutation which are commonly found in the Thai-Myanmar borders, *i.e.*, Mahidol, Viangchan, and Chinese 4 variants, were analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) according to the previously described method [13, 21]. The three specific primer pairs were used to amplified each variant; 5'-GCGTCTGAATGATGCAGCTCTGAT-3' and 5'-CTCCACGATGATGCGGTTCAACC-3' for Mahidol variant, 5'-TGGCTTTCTCTCAGGTCTAG-3' and 5'-GTCGTCCAGGTACCCTTTGGGG-3' for Viangchan and 5'-GGACTCAAAGAGAGGGGCTG-3' and 5'-GAAGAGGCGGTTGGCCGGTGAC-3' for Chinese variant 4 variant. Two microliter of DNA was

mixed, in 20 µl reaction, with 50 ng of each primer, 200 µM each dNTP, 1.5 mM $MgCl_2$, 1x buffer with KCl and 0.1 U of Taq polymerase (Fermentas). The PCR conditions used were as follows: 1 cycle of 5 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 58°C, and 1 min at 72°C; and final extension of 10 min at 72°C. PCR fragments were digested with appropriate enzymes and analyzed on 3% agarose gel containing ethidium bromide (Table 1) [13, 21].

RESULTS

The results of PCR-RFLP analysis of the common three synonymous G6PD variants, Mahidol, Viangchan, and Chinese 4 in 109 malaria patients are presented in Figure 1. Only Mahidol variant was found in 10 samples (9.17%), 1 in Thai (4.17%) and 9 in Burmeses (10.58%).

DISCUSSIONS

In this study samples collected from the Thai-Myanmar border, Mahidol G6PD variant was the only variant found. Mae Hong Son province in the northern region, Tak province in the western region, and Ranong province in the southern region are the three top 10 provinces of Thailand with malaria incidence in 2014. Most of the patients participated in the study were Burmese (85/109, 80%) and the prevalence rate of *P. vivax* infection was found to be higher than *P. falciparum* infection with the ratio 2.5:1.

In Southeast Asia, the prevalence of G6PD deficiency variants differs widely among each country and ethnic groups. In Myanmar, the most common variant is Mahidol variant [16]. In Thailand, the prevalence of G6PD mutations is approximately up to 20% but difference in each region; the Mahidol variant predominates in the western region bordering Myanmar, whereas the Viangchan variant predominates in the eastern region [22, 13]. Population migration across the two borders may well explain the high prevalence of Mahidol variant in Myanmar and the western region of Thailand [23, 14]. Previous study in a malaria endemic area of Thailand showed that two (Mahidol and Kaiping) and seven (Mahidol, Viangchan, Chinese 4, Union, Canton, Kaiping and Gaohe) G6PD variants were observed in samples collected from Burmese and Thai population, respectively [14]. The most predominant found in total population are Mahidol variant. The prevalence of G6PD variants in other malaria endemic regions should be performed together with correlation analysis for the possible link between G6PD variants and malaria disease. The information provided would be useful for consideration of the clinical use of primaquine as well as other 8-aminoquinoline in both *P. falciparum* and *P. vivax* infection.

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